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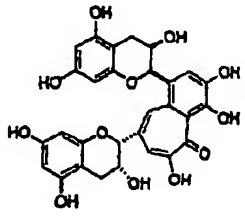
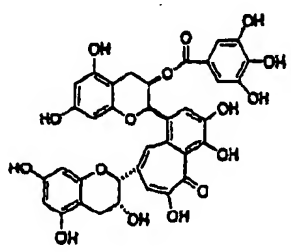
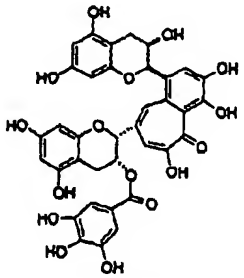
(54) Title: METHODS OF MAKING AND USING THEAFLAVIN, THEAFLAVIN-3-GALLATE, THEAFLAVIN-3'-GALLATE AND THEAFLAVIN 3,3'-DIGALLATE AND MIXTURES THEREOF



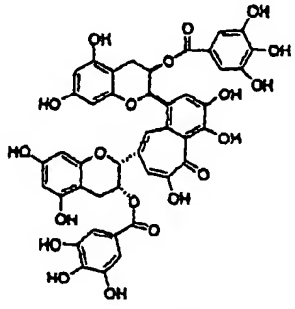
Theaflavin



Theaflavin-3-gallate



Theaflavin-3'-gallate



Theaflavin-3,3'-gallate

(57) Abstract: The present invention discloses methods of making a mixture of theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate, pharmaceutical compositions of the above mixture of theaflavins, diet supplement compositions of the above mixture of theaflavins and methods for using the above mixtures of theaflavin and pharmaceutical compositions thereof to treat or prevent various diseases. The present invention also discloses methods of making theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate, each as a separate compound, pharmaceutical compositions of the above compounds, diet supplement compositions of the above compounds and methods for using the above compounds to treat or prevent various diseases.

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METHODS OF MAKING AND USING THEAFLAVIN, THEAFLAVIN-3-GALLATE, THEAFLAVIN-3'-GALLATE AND THEAFLAVIN 3,3'-DIGALLATE AND MIXTURES THEREOF

Field Of The Invention

The present invention discloses methods of making a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate, pharmaceutical compositions of the above mixture of theaflavins, diet supplement compositions of the above mixture of theaflavins and methods for using the above mixtures of theaflavin and pharmaceutical compositions thereof to treat or prevent various diseases. The present invention also discloses methods of making theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate, each as a separate compound, pharmaceutical compositions of the above compounds, diet supplement compositions of the above compounds and methods for using the above compounds to treat or prevent various diseases.

Background Of The Invention

Tea, which is the most widely consumed beverage in the world other than water, is produced from the leaves of *Camellia Sinensis* and contain significant amounts of flavanoid compounds. Theaflavins, which comprise a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate, as depicted in Figure 1, are typically formed *via* polymerization of green tea polyphenols (*i.e.*, flavanoids) during fermentation of green tea to black tea. Typically, the concentration of theaflavins in black tea is between about 0.4% to about 1.8% by weight with the concentration of theaflavins in green tea usually being far less.

Theaflavins are responsible for the characteristic color (*i.e.*, brightness) and flavor (*i.e.*, briskness) of black tea. Flavanoids are effective anti-oxidants (Leung *et al.*, *J Nutr* **2001**, 131(9):2248-51; Sarkar *et al.*, *Biochem Biophys Res Commun* **2001**, 284(1):173-8; Yoshino *et al.*, *Biol. Pharm Bull.* **1994**, 17(1) 146-149) and may be efficacious against various diseases such as cancer, cardiovascular and cerebrovascular diseases, diabetes, *etc.* Further, flavanoids may possess significant anti-inflammatory antimicrobial and antiviral activity.

However, a significant problem in using theaflavins to treat or prevent various diseases is isolating theaflavins, either as a mixture or as individual compounds, in sufficient quantities, from naturally occurring sources using an economical procedure. Thus, what is needed are methods for isolating theaflavins, either as a mixture or as individual compounds, in sufficient quantities in a cost effective manner so that theaflavins, either as a mixture or as individual compounds, may be used to treat or prevent various diseases.

Summary Of The Invention

The present invention satisfies these and other needs by providing methods of making a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate and methods of making theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate, each as a separate compound and methods of using either the mixture of theaflavins or individual theaflavins to treat or prevent various diseases. Importantly, the methods above are efficiently carried out on large scale at minimal cost.

In one aspect, the current invention provides a method of making a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate. Green tea leaves are contacted with an aqueous buffer to form a reaction mixture. The reaction mixture is then contacted with oxygen to begin fermentation and is fermented for a time sufficient to form the mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate. Fermentation is then terminated and the reaction mixture is separated from the mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3' digallate.

In another aspect, the present invention provides pharmaceutical compositions comprising the mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate or a pharmaceutically available salt, hydrate or solvate thereof. The pharmaceutical compositions generally comprise the mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3'-digallate or a pharmaceutically available salt, hydrate or solvate thereof and a suitable excipient, carrier or diluent. The composition may be formulated for veterinary uses or for use in humans.

In still another aspect, the present invention provides diet supplement compositions comprising the mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate. The diet supplement compositions generally comprise the mixture

38. A method of treating or preventing dementia and physical disorder induced by cardio- and cerebral-vascular disease comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of a mixture comprising theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate or a pharmaceutically available salt, hydrate or solvate thereof.

39. A method of treating or preventing dementia and physical disorder induced by cardio- and cerebral-vascular disease comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of the pharmaceutical composition of Claim 13.

40. A method for making theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate, each as a separate compound, said method comprising the steps of:

contacting tea polyphenols with a aqueous buffer and polyphenol oxidase to form a reaction mixture;

contacting the reaction mixture with oxygen to begin fermentation;

fermenting the reaction mixture for a time sufficient to form a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate;

terminating fermentation; and

separating the reaction mixture to provide theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate or theaflavin-3,3'-digallate, each as a separate compound.

41. The method of Claim 40, wherein the separating step further comprises:

contacting the reaction mixture with an organic solvent;

contacting the solvent with dilute aqueous base;

separating the solvent from the base; and

contacting the solvent with a chromatographic media; and

eluting the theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin 3,3' digallate, each as a single compound, from the chromatographic media.

42. The method of Claim 41 wherein solids are removed from the reaction prior to contacting with the organic solvent.